

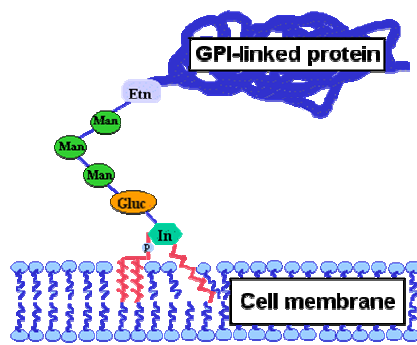
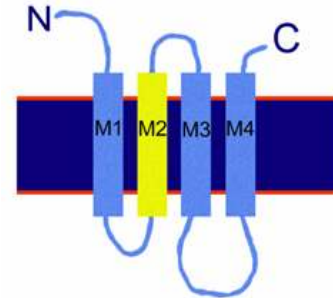
Membrane Protein Anchorage

Aim

To determine the form of membrane anchorage used in mammalian versions of alkaline phosphatase and aminopeptidase N.

Background

Membrane proteins are typically bound to the membrane through having one or more trans-membrane helices as part of the structure of the membrane. These hydrophobic areas span across the membrane and hold the protein within the membrane.



Proteins

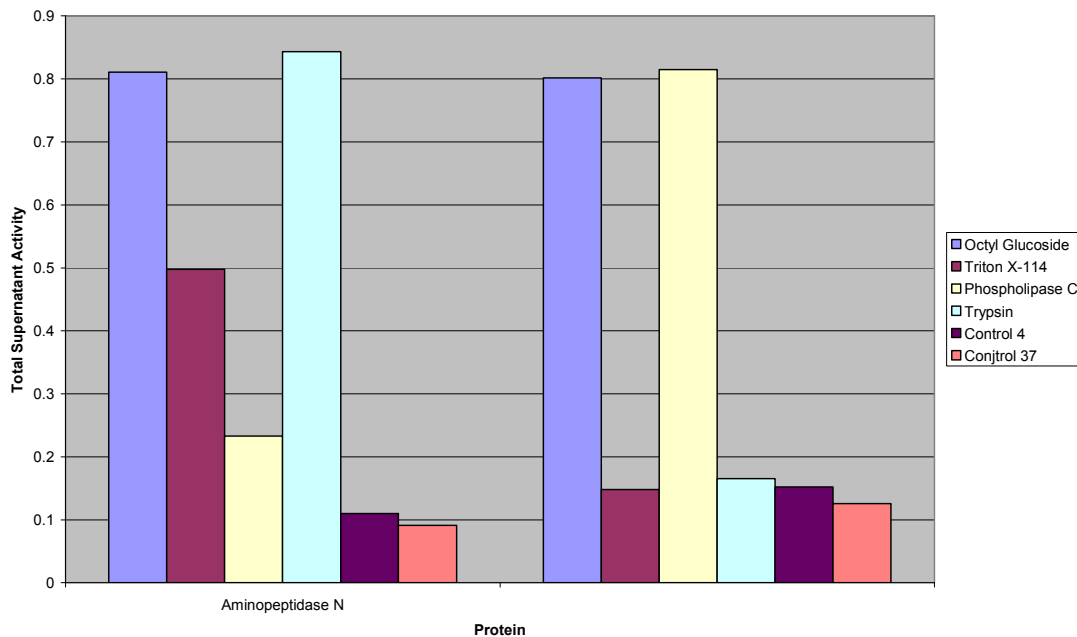
can also however be attached to the membrane through what is known as a GPI (glycosyl-phosphatidylinositol) anchor. This is effectively four amino acids (three manose and a glucosamine) in a sugar structure, attached to a phospholipids. The phospholipids resides in the membrane and the C terminus of the protein is covalently bound to the end of the anchor.

Results

Triton X100 PAGE:

405nm Absorbance:

	Pellet	Activity					
		Aminopeptidase N		Total		Alkaline Phosphatase	
		Supernatan	Supernatant	Pellet	Supernatan	Supernatant	
Octyl							
Glucoside	0.113	0.485	0.811037	0.124	0.50125	0.801679	
Triton X-114	0.3745	0.3715	0.497989	1.1535	0.20075	0.148237	
Phospholipase							
C	0.524	0.15925	0.233077	0.18875	0.83	0.814724	
Trypsin	0.099	0.53375	0.84354	0.5415	0.107	0.164996	
Control 4	0.831	0.1025	0.109802	0.893	0.16025	0.152148	
Conjntrol 37	0.701	0.0705	0.09138	0.70125	0.101	0.125896	



Conclusions

Phospholipase C cleaves proteins at phosphatidylinositol groups. This means that GPI anchored proteins are separated from their phospholipids anchor and are free in solution. Proteins that are still GPI anchored but removed from the membrane however are caused to form micelles (spherical multi-molecular structures) because the hydrophobic fatty-acid tails of the attached phospholipid needs to be kept separated from the water. These micelles pack all the tails together. This means that when GPI anchored proteins are subjected to a non-denaturing pages, then those that are cleaved will move down quickly while the uncleaved will struggle to move through the gel while in bulky micelles. It can be seen here that Alkaline Phosphotase is in fact GPI anchored (or at least contains a hydrophobic tail that is cleaved by Phospholipase C.

The activity results show a large difference in behaviour when phospholipase C is used – Alkaline Phosphotase has very high supernatant activity suggesting large amounts of Alkaline Phosphotase were released into solution when the GPI anchor was cleaved, while Aminopeptidase N seems to have been almost unaffected. This suggests that Aminopeptidase N is not GPI anchored.

Use of Triton X114 on the two proteins shows a significant difference in behaviour too – the Alklaline Phosphotase appears barley solubilised by it, as would be expected for a GPI protein, whereas the Aminopediase N shows a quite significant amount being present in the supernatant, suggesting it is solubilised by the Triton.

Trypsin cleaves proteins after certain hydrophobic amino acids (lysine and arginine). This action would separate a trans-membrane anchored protein into the soluble part and the transmembrane section, however would have no notable effect on a GPI anchored protein. We can see in the results that use of trypsin results in a significant amount of protein in the supernatant for Aminopeptidase N but almost none of Alkaline Phosphotase.

Based on the combination of these results, we can postulate that Aminopetidase N is transmembrane anchored, but the functional part of the protein is soluble and that Alkaline Phosphatase is GPI anchored into the membrane.